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begin model
begin parameters
    Temp                37                #Temperature of reaction. Affects rate of intein splicing
    ppt                 1000              #Total parts per trillion of estrogen
    rfp_copies          15                #Number of copies of rfp containing plasmid per cell
    int_copies          2984              #Number of copies of intein containing plasmids per cell
    estrogen_out      ppt * 5.6e-02      #Total concentration of estrogen outside cell
    estrogen_in        0                  #Total concentration of estrogen molecules inside cell
    t7_intein           0                  #Total concentration of t7-intein complexes
    t7_estrogen_u       0                  #Total concentration of unspliced T7-estrogen complexes
    t7_estrogen_s       0                  #Total concentration of spliced T7-estrogen complexes
    t7_nc               0                  #Total concentration of completed t7 polymerase
    plas_int            int_copies * 1.0e-03 #Total concentration of T7-intein plasmids
    plas_rfp            rfp_copies * 1.0e-03 #Total concentration of rfp plasmid
    t7_rfp              0                  #Total concentration of t7 bound to plasmid
    mrna_rfp            0                  #Total concentration of mRNAs to code for RFP
    prot_rfp            0                  #Total concentration of RFP proteins
    mrna_int            0                  #Total concentration of mRNAs to code for T7-Intein
    prot_yfp            0                  #Total concentration of YFP proteins

    k_e                1.7e-02            #Rate at which estrogen diffuses through membrane
    k_e_t7_on           1.3e-03            #Rate at which estrogen binds to T7 intein
    k_e_t7_off          1.2e-03            #Rate at which estrogen dissociates from T7 intein
    k_splice            7.1e-04            #Rate at intein splices out
    k_t7_rfp_on         3.3e-01            #Rate at which T7 binds to RFP plasmid
    k_t7_rfp_off        1.0e-01            #Rate at which T7 dissociates from RFP plasmid
    k_rna_rfp           8.8e-01            #Rate at which rna is synthesized from RFP plasmid
    k_rna_deg_rfp       4.3e-03            #Rate at which rna of RFP degrades
    k_prot_rfp          9.0e03             #Rate at which RFP protein is made
    k_prot_deg_rfp      8.3e-04            #Rate at which RFP proteins degrades
    k_rna_int           2.6e-02            #Rate at which rna is synthesized from T7-Intein plasmid
    k_rna_deg_int       4.3e-03            #Rate at which rna of T7-intein degrades
    k_prot_int          2.2e-03            #Rate at which T7-intein is made from mRNA
    k_prot_deg_int      9.7e-04            #Rate at which T7 degrades
end parameters

begin molecule types
    E(I~U~B,L~I~O)      #Estrogen outside of cell, can be inside or outside cell
    T7_intein(n,r,Y~A~S,c) #T7 intein. Can be attached or spliced
    T7_n(n,b)            #N-terminal t7
    T7_c(c)              #C-terminal t7
    RFP_plasmid(p,A~0~1) #Plasmid that codes for RFP
    mRNA_RFP()           #mRNA that codes for RFP
    protein_RFP()        #RFP protein
    INT_plasmid()        #Plasmid that codes for T7-Intein and YFP
    mRNA_INT()           #mRNA that codes for T7-Intein and YFP
    protein_YFP()        #YFP protein
end molecule types

begin seed species
    E(I~U,L~O)           estrogen_out
    E(I~U,L~I)           estrogen_in
    T7_n(n!1,b).T7_intein(n!1,r,Y~A,c!2).T7_c(c!2) t7_intein

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T7_n(n!1,b).E(!3~B,L~I).T7_intein(n!1,r!3,Y~A,c!2).T7_c(c!2)    t7_estrogen_u
E(!3~B,L~I).T7_intein(n,r!3,Y~S,c)                                t7_estrogen_s
T7_n(n!4,b).T7_c(c!4)                                              t7_nc
RFP_plasmid(p,A~0)                                                  plas_rfp
RFP_plasmid(p!5,A~1).T7_n(n!4,b!5).T7_c(c!4)                    t7_rfp
mRNA_RFP()                                                          mrna_rfp
protein_RFP()                                                       prot_rfp
INT_plasmid()                                                       plas_int
mRNA_INT()                                                          mrna_int
protein_YFP()                                                       prot_yfp
end seed species

begin observables
Molecules    E_out    E(!~U,L~O)
Molecules    E_in     E(!~U,L~I)
Molecules    T7_unbound    T7_n(n!1,b).T7_intein(n!1,r,Y~A,c!2).T7_c(c!2)
Molecules    T7_estrogen_u    T7_n(n!1,b).E(!3~B,L~I).T7_intein(n!1,r!3,Y~A,c!2).T7_c(c!2)
Molecules    T7_estrogen_s    E(!3~B,L~I).T7_intein(n,r!3,Y~S,c)

Molecules    T7_nc    T7_n(n!4,b).T7_c(c!4)
Molecules    RFP_Plas_U    RFP_plasmid(p,A~0)
Molecules    T7_RFP    RFP_plasmid(p!5,A~1).T7_n(n!4,b!5).T7_c(c!4)
Molecules    mRNA_RFP    mRNA_RFP()
Molecules    prot_RFP    protein_RFP()
Molecules    INT_Plas    INT_plasmid()
Molecules    mRNA_INT    mRNA_INT()
Molecules    prot_YFP    protein_YFP()
end observables

begin functions
mRNA_RFP_synth() = k_rna_rfp * T7_RFP
mRNA_RFP_deg() = k_rna_deg_rfp
protein_RFP_synth() = k_prot_rfp * mRNA_RFP * sqrt(1/(1 + prot_RFP))
mRNA_INT_synth() = k_rna_int * INT_Plas
protein_INT_synth() = k_prot_int * mRNA_INT * sqrt(1/(1 + T7_unbound))
#Rate of YFP synth = Rate of intein synthesis
protein_YFP_synth() = k_prot_int * mRNA_INT * sqrt(1/(1 + prot_YFP))
int_Splice() = k_splice * 1/(1 + sqrt(abs(37 - Temp)))
e_in() = k_e * (E_out)/(E_out + E_in)
e_out() = k_e * (E_in)/(E_out + E_in)
end functions

begin reaction rules
#Describes Rate at which mRNA T7-Intein is transcribed/degraded
0 <-> mRNA_INT()                                mRNA_INT_synth(), k_rna_deg_int

#Describes Rate at which T7-Intein is translated/degraded
0 <-> T7_n(n!1,b).T7_intein(n!1,r,Y~A,c!2).T7_c(c!2)    protein_INT_synth(), k_prot_deg_int

#Describes Rate at which T7 polymerase is degraded
T7_n(n!4,b).T7_c(c!4) -> 0                                k_prot_deg_int

#Describes estrogen entering and leaving cell

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E(I~U,L~O) <-> E(I~U,L~I)

e_in(), e_out()

#Describes rate of T7-estrogen association/disassociation

E(I~U,L~I) + T7_n(n!1,b).T7_intein(n!1,r,Y~A,c!2).T7_c(c!2) <->

T7_n(n!1,b).E(I!3~B,L~I).T7_intein(n!1,r!3,Y~A,c!2).T7_c(c!2)

k_e_t7_on, k_e_t7_off

#Describes the rate at which the intein is spliced out and t7 is formed

T7_n(n!1,b).E(I!3~B,L~I).T7_intein(n!1,r!3,Y~A,c!2).T7_c(c!2) ->

E(I!3~B,L~I).T7_intein(n,r!3,Y~S,c) + T7_n(n!4,b).T7_c(c!4)

int_Splice()

#Describes the rate at which T7 binds to promoter of plasmid 2

T7_n(n!4,b).T7_c(c!4) + RFP_plasmid(p,A~0) <->

RFP_plasmid(p!5,A~1).T7_n(n!4,b!5).T7_c(c!4)

k_t7_rfp_on, k_t7_rfp_off

#Describes the rate at which the mRNA of RFP is synthesized/degraded

0 <-> mRNA_RFP()

mRNA_RFP_synth(), mRNA_RFP_deg()

#Describes the rate at which RFP is synthesized/degraded

0 <-> protein_RFP()

protein_RFP_synth(), k_prot_deg_rfp

#Describes the rate at which YFP is synthesized

0 <-> protein_YFP()

protein_YFP_synth(), k_prot_deg_rfp

end reaction rules

end model

actions

#construct reaction network

generate_network({overwrite=>1})

#kinetics

saveConcentrations();

simulate({method=>"ode",suffix=>"ssa",t_end=>7200,n_steps=>7200,atol=>1e-12,rtol=>1e-12,sparse=>1})

resetConcentrations();